Significance of the leaf epidermis fingerprint for taxonomy of Genus *Rhododendron*

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Abstract: Leaf epidermal fingerprints of six species of *Rhododendron*（*Rh. Aureaum*, *Rh. dauricum*, *Rh. micranthum*, *Rh. Mucronulatum*, *Rh. Redowskianum*, *Rh. schlippenbachii*）were observed by optical microscope with nail polish expression method in Key Laboratory of Forest Plant Ecology of Ministry Education China in Northeast Forestry University in 2004. The leaf morphological features including of stomata types, characters of guard cells, subsidiary cells in lower epidermis were observed. And ordinary cells (in shape and anticlinal walls feature) as well as the trichomes in both sides of the leaves are described in detail. The results showed that there were three types of stoma in six investigated *Rhododendron* species, from which Pericytic stomata type exists in three species (*Rh. dauricum*, *Rh. micranthum*, and *Rh. mucronulatum*), Anomocytic stomatal type in *Rh. Redowskianum*, Diacytic stomata type in *Rh. aureaum* and *Rh. schlippenbachii*. The subsidiary cells of the Pericytic and Diacytic stomata type are different in shape and surface feature between the species, respectively. The ordinary epidermal cells show a variety from quadrangular to hexagonal, polygonal or irregular in surface view, the anticlinal walls are straight or sinuose. Trichomes (gland scales) are present in the both of the leaf sides in three species (*Rh. dauricum*, *Rh. micranthum*, and *Rh. mucronulatum*). All of these detail leaf features show specific specificity of leave finger print for 6 rhododendrons.

Keywords: Taxonomy Significance; Epidermal Fingerprint; Stomata; Trichome; *Rhododendron*.
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Introduction

Rhododendron (Ericaceae) is considered as a large genus (Hu *et al*. 1994; Fang 1999) because of having more than 850 different natural species (Zhang 2003), of which 8 species distribute in northeast of China (Liu *et al*. 1955). These species are native to the temperate regions of Asia, North America, and Europe, as well as to the tropical regions of Southeast Asia and northern Australia, largest number of which are native to Asia, where they occur in a variety of habitats, and exhibit an enormous diversity in size and shape**.**

A classification system of rhododendron first imposed by Linnaeus in 1764 has been developed and is also constantly being revised as new discoveries appear. New discoveries lead botanists to refine the classification system of the plants. Many studies have been conducted on classification of rhododendrons by morphological characteristics, such as leaf, flower, trichome (gland scale), seed, and so on (Hu *et al*. 1994; Fang 1999; Hedegaard 1980a, 1980b). Now the genus *Rhododendron* has systematic problems at various classification levels, owing to the enormous number of species and conflicting ideas of classification proposed by many taxonomists (Kurashige 2001). Developing new approaches to identify the species characteristics is necessary for rhododendron taxonomy.

This article for cuticular analysis provides a proof of biological classification. The taxonomic significance of epidermal morphology is well documented in botanical literature (Dehgan 1980). Some particular groups of plants or taxa seem to be characterized by specific type of epidermal features, which are the epidermis, stomata, gland and trichomes (Park 1994; Hong *et al*. 1999; Hong *et al*. 2000). Many distinct patterns of stomata have been found in epidermis of different plants. Stomata in epidermal morphology can be considered as a classified feature depending on patterns of stomata and subsidiary cells in a particular plant (Metcalfe 1950; Shtromberg 1956; Van Cotthem 1970b; Fryns-Claessens *et al*. 1973; Rasmussen 1981; Prabhakar 2004). The peculiar types of stomatal in dicotyledons have been reported in various families such as in Acantheceae (Paliwal 1966), Leguminosaea (Edeoga *et al*. 1996), Nyctaginaceae (Edeoga *et al*. 2001) and Commelinaceae (Edeoga *et al*. 2001). Stomatal characteristic, dermal appendages have often been used as morphological markers for plant taxonomy (Paliwal 1969a; Van Cotthem 1970a; Guyot 1971; Leelavathi 1980; Jelani *et al*. 1990; Ferzana *et al*. 1991).

Epidermal cells may potentially adopt certain fates through a cell lineage based mechanism or a cell interaction mechanism (Glover 2000). The ordinary epidermal cells can vary in shape, size, arrangement and anticlinal wall (straight or sinuose, thin or thick) in different species. Thus, it is possible that different species of plant show specific fingerprints due to their different cell lineages.

Leaf epidermal fingerprint consists of different typical cells, not only specialized cells, such as stomatal complex cells (guard cells and subsidiary cells, if present), trichome and other different kinds of epidermal appendages, but also a large number of ordinary cells. We have a hypothesis that these various types of epidermal cells combine in different ways in different species and show specific specificity which may be considered as indicators for classification of plant species.

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However, so far no detailed epidermal morphological studies have been conducted on all of rhododendron species though some researchers had done some anatomical researches for some other species of this genus (Copeland 1943; Cox 1948). The main objective of the present work is to investigate and describe the epidermal micromorphological fingerprint of 6 *Rhododendron* species in order to provide more detailed descriptions to discuss their taxonomic significance.

Material and Methods

Material

Mature leaf materials used for this study were collected from living trees and herbarium specimens (Table 1). Voucher specimens of 6 species are deposited in the Herbarium of Key Laboratory of Forest Plant Ecology of Ministry Education China at Northeast Forestry University (HNEFU). The six species (*Rhododendron aureum* Georgi., *Rh. dauricum* Linn., *Rh. micranthum* Turcz., *Rh. mucronulatum* Turcz., *Rh. redowskianum* Maxim. and *Rh. schlippenbachii* Maxim.) were native in Northeast of China, Korea, Far East of Russia and two of them in Japan (*Rh. dauricum*, *Rh. mucronulatum*).

Table 1. A list of the studied species and their origin

Species	Collecting place		Specimen Number*
	Specimen	Fresh leaf	
Rh. aureum	HNEFU,		8649
Rh. dauricum	HNEFU	HCFPG DM 600 m a.s.l. DM, 700m a.s.l.	8654
Rh. micranthum	HNEFU	HCFPG	Living plant introduced from Dangong in 2003
Rh. mucronulatum	HNEFU	HCFPG	Living plant introduced from Dangong in 2003
Rh. redowskianum	HNEFU		1616
Rh. schlippenbachii	HNEFU		20294

Notes: HNEFU--- Herbarium of Key Laboratory of Forest Plant Ecology of Ministry Education China at Northeast Forestry University; HCFPG----Harbin City Forest Plant Garden; DM---Daxxing'an Mountain

*Voucher specimens of the plants are deposited in the HNEFU

Epidermal impressions

The epidermal impressions were made with nail polish method. Leaf portions between second-order veins of leaf in width of 0.5−1.0 cm from the middle were selected respectively. The two sides of the leaf samples were painted with clear nail polish respectively. After the nail polish had dried thoroughly, a square of very clear strip was selected and was gently peeled from the leaf completely and attached to a clear plastic package tape piece, and then a leaf impression was made. The leaf impressions were taped to a clean glass slide respectively for observation under microscope.

Observation

Leaf impression was observed for the lower and upper leaf surface with a light microscope (Olympus BH-2, Japan) fitted with a digital camera (Olympus C-5060) at a magnification of 200 and 400 times. For accuracy, several fields of the different leaf impressions were examined. Observed characteristic parameters are shape, arrangement, Anticlinal wall features of ordinary epidermal cell, stomatal complex (guard cells and subsidiary cells), trichome (gland scale).

The terms about stomatal features used in this study are according to the nomenclature and classification by Metcalfe (1950) and Prabhaka (2004) mainly as follows.

Stomatal pore: An opening in the epidermis surrounded by a pair or more guard cells.

Stoma: Stomatal pore and a pair of guard cells. Stomatal complex: stoma surrounded by subsidiaries (stomatal types).

Subsidiary cells: cells surrounding a pair of guard cells in one or more cycles; subsidiaries of the cycle abutting on stoma may or not be distinct from the adjacent epidermal cells.

Results

The observation result shows that, the upper epidermises of the leaves consist of ordinary epidermal cells in the six species. Stomatal complex distributed in lower epidermis is found in all of the 6 species and shows paracytic type, anomocytic type and diacytic type, respectively. The pavement epidermal cells and stomatal complex combine in different ways in different species and show specific conserved in the six *Rhododendron* species (Table 2). Trichom (gland scale) was found in three of the species (*Rh. dauricum*, *Rh. micranthum*, *Rh. mucronulatum*) in the both side of the leaves.

Table 2. Epidermal Characteristics of six Rhododendron species

Species	Shape of Epidermal cell / anticlinal wall		Stomatal Type	Trichome Type in both leaf sides
	Upper epidermis	Lower epidermis		
Rh. aureum	Lathy or elongated rectangular/ slight sinuos	Irregular / unclear	Diacytic	Absent
Rh. dauricum	Irregular / sinuos	Pentagona, quadrangular or hexagonal / slightly sinuos or nearly straight	Paracytic	Multicellular Circular shape in top view
Rh. micranthum	Nearly honeycomb / nearly straight	Quadrangular to polygonal/ near straight	Paracytic	Multicellular Circular shape in top view
Rh. mucronulatum	Quadrilateral, pentagonal or hexagonal / slight sinuos	Irregular / sinuos	Paracytic	Multicellular Circular shape in top view
Rh. redowskianum	Rectangular or square / near straight	Irregular long shaped / sinuos	Anomocytic	Absent-
Rh. schlippenbachii	Quadrilateral, pentagonal or hexagonal / straight	Irregular / sinuos	Diacytic	Absent-

Rh. aureaum

Lower epidermis: The ordinary epidermal cells are irregular shape and unsharp image (Fig. 1a). It may be owing to a thick cerolipoid layer covered in the epidermis. Stomata are broad elliptical in shape, and are considered as diacytic type according to Matcalfe and Chalk (1950) and Prabhakar (2004). A pair of guard cells is surrounded by two distinct subsidiary cells，which are the same in shape and size (Fig. 1a, Fig. 2a). Occasionally 2−3 stomata may become laterally contiguous (Fig. 2b).

Upper epidermis: The ordinary cells of the epidermis are small and arranged in irregular lathy in shape or appear to be elongated rectangular in surface view. The anticlinal walls are most slight sinuosity or near straight (Fig. 3a.).

Rh. dauricum

Lower epidermis: The most of ordinary epidermal cells appear to be pentagonal and rarely quadrangular or hexagonal in surface view. The anticlinal walls are most slight sinuosity or near straight (Fig.1b). Stomata are oblong elliptse or circule in shape, are considered as peracytic type according to Prabhakar (2004). In this type, a pair guard cells are surrounded by a single narrow subsidiary cell, irregularly shaped, which is indistinct from the guard cells (Fig. 2 d,e). But occasionally the guard cell surrounded by a few small subsidiary cells was found, which are indistinct from the guard cells. Occasionally 2-3 stomata may show laterally contiguous or polar contiguity. Rarely are the contiguous stomata arranged at right angles to each other (Fig. 2f) or irregularly. Gland scales are found in lower epidermis of *Rh.dauricum*. These gland scales distribute randomly and circularly shaped. The scale consists of two kinds of cells. The border cells are long shaped surround several central cells, which are in irregular shape and smaller than border cells (Fig. 2c).

Upper epidermis: the epidermal cells are arranged randomly, irregular in shape. The anticlinal walls are mostly sinuous. The similar Gland scales in lower epidermis distributed randomly and in upper epidermis also are found (Fig. 3b).

Rh. micranthum

Lower epidermis: the ordinary epidermal cells appear to be quadrangular to polygonal in shape. The anticlinal walls are near straight (Fig. 1c). There are lipoid drops accumulating in some of the cells (Fig. 2g). Stomata are elliptical in shape and show as Pericytic Type according to Prabhakar (2004). A pair of guard cells is surrounded by a single distinct narrow subsidiary cell, which are monocyclic multiangular (Fig. 2g) or radial in shape (Fig. 2h). Occasionally there is two or more subsidiary cells around the guard sells. Two stomata arrange laterally contiguous (Fig. 2i) or irregularly in direction, in which cellulose molecular bundles in radial are found. It may be owing to the cuticle layer thickening (Fig. 2g-i). Gland scales distribute in lower epidermis of *Rh. micranthum* randomly (Fig. 2l). The composition of these gland scales is similar with those of *Rh. duricum*.

Upper epidermis: the epidermis cells arrange regularly and the shape of the cells are similar with honeycomb shape. The anticlinal walls are clearly, honeycomb. Gland scales also distribute in lower epidermis (Fig. 3c).

Rh. mucronulatum

Lower epidermis: the ordinary epidermal cells appear to be irregular in shape. The anticlinal wall is sinuous (Fig. 1a). Stomata are elliptical in shape, Pericytic Type according to Prabhakar (2004). A pair of guard cells is surrounded by a single distinct narrow subsidiary cell, which are monocyclic irregular in shape. The subsidiary wall abutting against the stoma is indistinct (Fig.2j). Occasionally 2–3 stomata may become contiguous arranged irregularly (Fig. 2k). Gland scales are found in lower epidermis of *Rh.mucronulatum,* the composition of which is similar with those of *Rh. duricum*.

Upper epidermis: The epidermal cell arranged orderly relatively and appear to be quadrilateral, pentagonal or hexagonal in surface view. The anticlinal walls are straight and clear. Broad dark strips impresses are found in the cells (Fig. 3d). The similar gland scales are found in upper epidermis also (Fig. 3d).

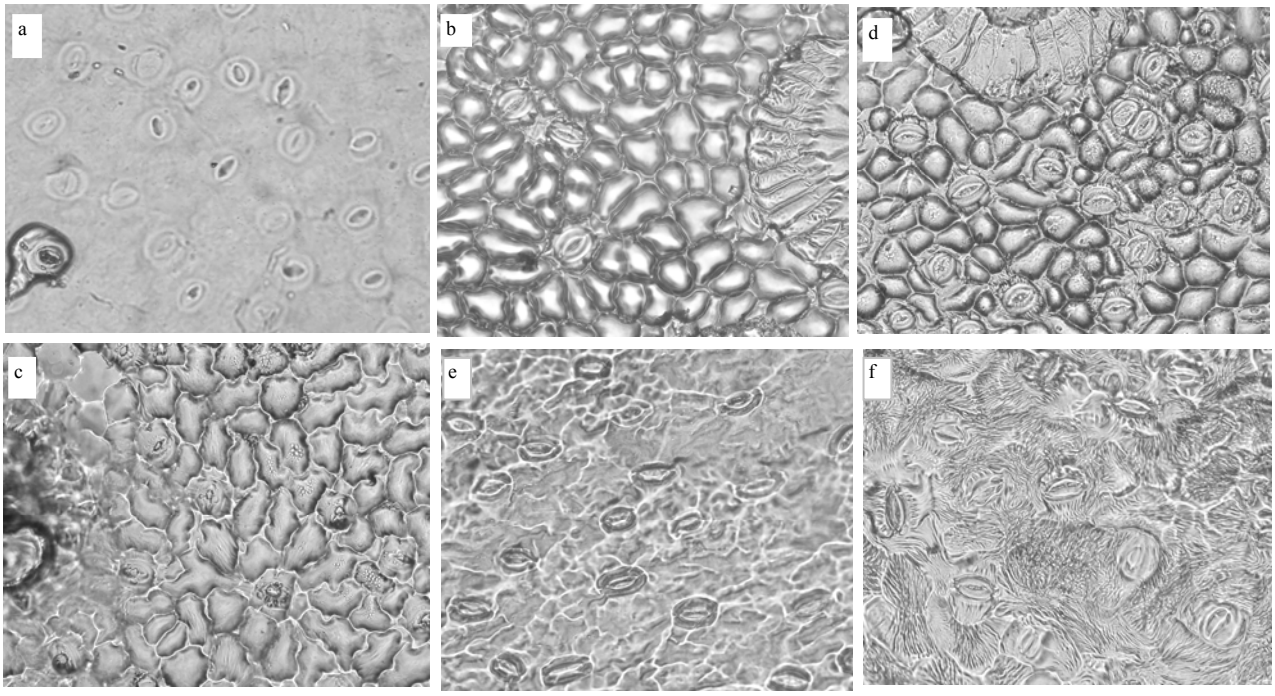

Fig. 1 Lower epidermis impressions of six *Rhododendron* species.

a. Ordinary epidermal cells, stomatal complex of *Rh. aureum* b. Ordinary epidermal cells, stomatal complex and cells group of a trichome of *Rh. dauricum*. c. Ordinary epidermal cells, stomatal complex and cells group of a trichome of *Rh. micranthum*. d. Ordinary epidermal cells, stomatal complex and cells group of a trichome of *Rh. mucronulatum*. e. Ordinary epidermal cells, stomatal complex of *Rh. redowskianum*. f. Ordinary epidermal cells, stomatal complex of *Rh. schlippenbachii*.

Rh. redowskianum

Lower epidermis: The ordinary epidermal cells are irregular pattern in shape, but generally showed long shape. The anticlinal walls are sinuous (Fig. 1e). Stomata are oblong in shape, are considered as nomocytic type according to Matcalfe and Chalk (1950), in which the guard cells are surrounded by a certain number of cells that do not differ in size and shape from other epidermal cells (Fig. 2m). The stomata are oriented in the direction to extend parallel to lateral vine of the leaf (Fig. 1f).

Upper epidermis: The epidermal cells arrange orderly and appear to be rectangular or square in surface view. Generally two cells arranged in lateral direction and two in longitudinal direction alternately. The anticlinal walls are near straight (Fig. 3e).

Rh. Schlippenbachii

Lower epidermis: the ordinary epidermal cells are irregular in shape. Dark sinuate leptonema strias impress are allover densely in the cells (Fig. 1f). Stomata are elliptical in shape. The stomatal type is Diacytic type (Matcalfe and Chalk 1950, Prabhakar 2004). The two abutting subsidiaries cells, which are not the same in shape and size (showed no conjoint walls towards both poles), indistinct, parallel to the guard cell, and subsidiary cells conjoined with the guard cells with distinct radial cellulose molecular bundles (Fig. 2n). 2–3 stomata may become laterally contiguous or may show polar contiguity or irregularly arranged each other (Fig .2o).

Upper epidermis: the epidermal cells arrange closely and appear to be quadrilateral, pentagonal or hexagonal in surface view. Intercellular spaces are clearly. Cell anticlinal walls are straight. Sinuate leptonema strias impress are allover densely in the cells.

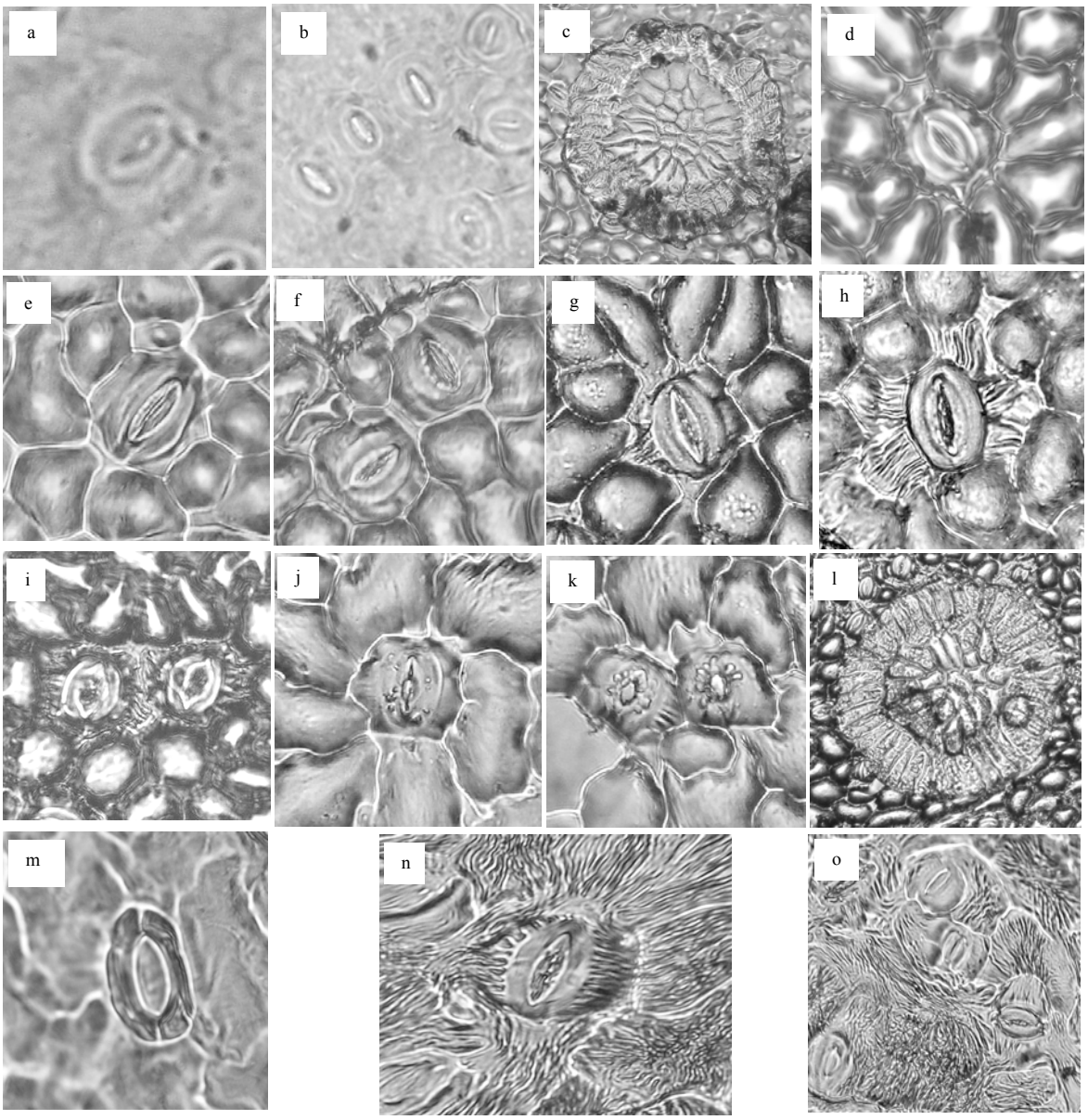

Fig. 2 Stomatal complex and trichome impressions of six species *Rhododendron.*

a. stomata and b. laterally and irregularly contiguous stomata of *Rh. aureaum*. c. trichome in lower epidermis in top view. d. e. Stomatal complex. f. two perpendicularly contiguous stomata of *Rh. dauricum.* g,h. Stomatal complex. i. two laterally contiguous stomata of *Rh. micranthum.* j. stomatal complex, k. two laterally contiguous stomata and l. trichome in lower epidermis of *Rh. mucronulatun* in top view. m. stomata of *Rh. redowskianum*. n. stomatal

complex and o. four contiguous stomata of *Rh. schlippenbachii*.

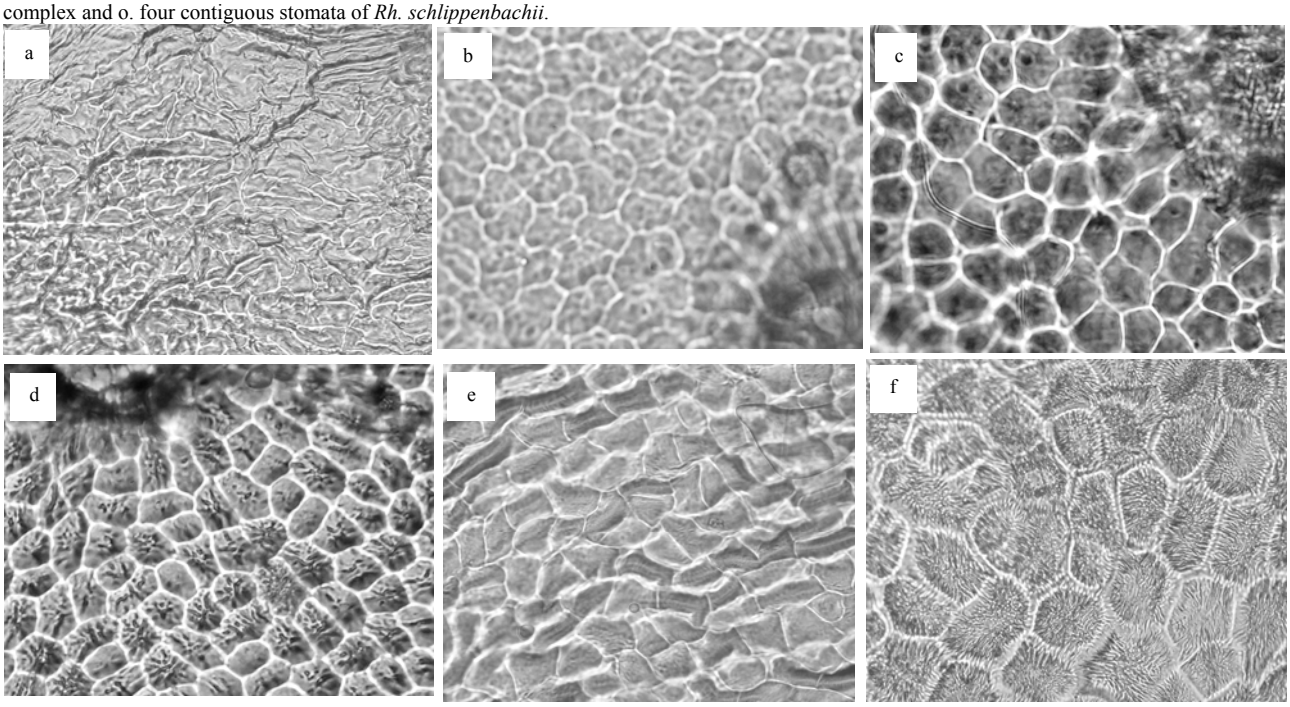

Fig.3 Upper epidermis impressions of six *Rhododendron* species.

a. Ordinary epidermal cells of *Rh. aureum* b. Ordinary epidermal cells and gland scale cells group of *Rh. dauricum*. c. Ordinary epidermal cells and gland scale cells group of *Rh. micranthum*. d. Ordinary epidermal cells and gland scale cells group of *Rh. mucronulatum*. e. Ordinary epidermal cells of *Rh. redowskianum*. f. Ordinary epidermal cells of *Rh. schlippenbachii*.

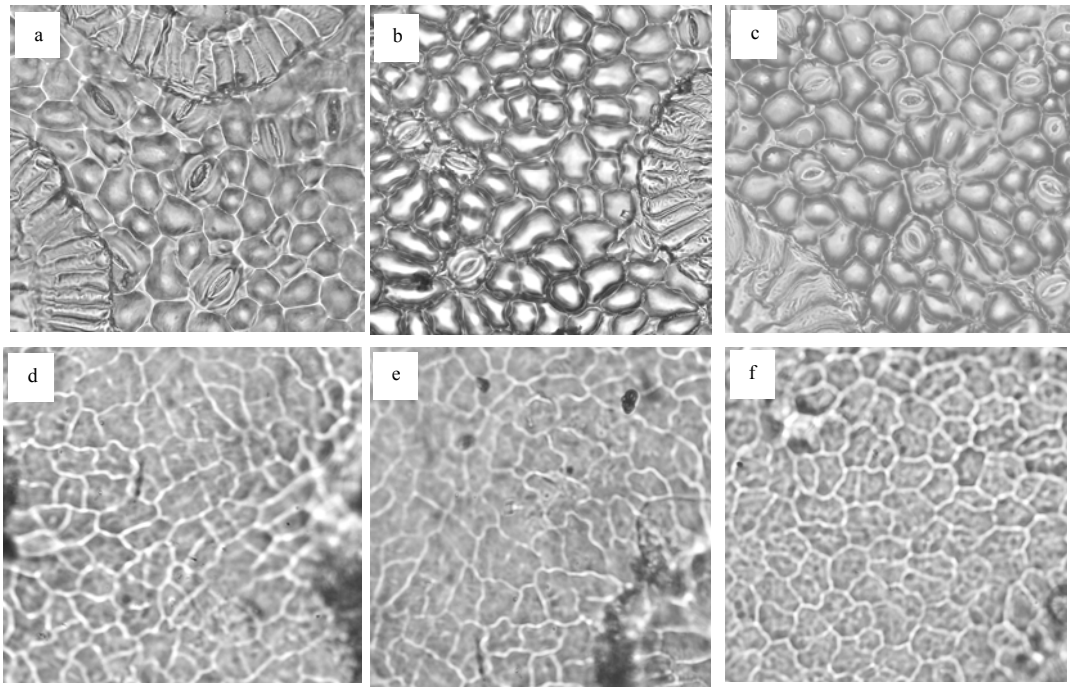

Fig.4 Leaf epidermis impressions of *Rhododron dauricum* from three populations distributed in different locations.

a, d. Show the lower epidermis and upper epidermis from Daxingan mountain (51°N,124°E, 600m hight), respectively. b, e. Showing the lower epidermis and upper epidermis from Daxingan mountain (51°N, 124°E, 700 m at height). c,f. Showing the lower epidermis and upper epidermis from Harbin city

Discussion

The epidermal fingerprint analysis in this work provides the first detailed description of leaf features for six species of *Rhododendron*. The results show distinct different epidermal fingerprints among the six species. The features of ordinary epidermal cells, guard cells, subsidiary cells and trichones show specific specificities and have significance for classification of *Rhododendron* in species level.

According to the morphological classification by Nakai (1917), the investigated six species belong to 5 sections respectively, which are section Osmothumnus Maxim. (*Rh. micranthum*), section Rhodorastrum Maxim.(*Rh. dauricum*, *Rh. mucronulatum*), Section Therorhodion (Maxim.) Drude.(*Rh. redowskianum*), section Eurhododendron (DC.)A. Gray. (*Rh. aureaum*) and section Azalea (Linn.) Maxim. (*Rh. schlippenbachii*). The obtained epidermal impression features of the 6 species are coincident with morphological classification. *Rh. dauricum*, and *Rh. mucronulatum* have similar epidermal fingerprint (stomata are Pericytic type, subsidiary cells are similar in shape (Fig. 2d,j)) comparing with other four species, indicating the close relationship in the same section. The epidermal fingerprint of *Rh. micranthum* is similar with that of *Rh. dauricum* and *Rh. mucronulatum* in a certain extent (stomata is Pericytic type, ordinary cells in lower epidermis are similar with those of *Rh. dauricum* in shape). It is coincident with morphological classification that the three species have gland scales in both sides of the leaves.

Among the examined six species, three of them is peracytic somata type (*Rh. dauricum*, *Rh. micranthum*, and *Rh. mucronulatum*), 2 of them is Diacytic type *(Rh. aureaum and Rh. Schlippenbachii).* But the subsidiary cells of them are very different in shape, size and the surface feature in same stomatal type. So these detail characters should be considered as indicators of natural taxonomic affinity for the taxonomical study of *Rhododendron*.

Considering the possibility of ecological effect on the leaf features of the same species between different populations, a comparative study has been done for the leaf fingerprint between three populations of *Rh. dauricum*, distributing in Daxingan Mountain (51°N, 124°E, at latitude of 500 m and 700 m) and in Harbin city (45°N, 127°E, at latitude of 400 m), respectively. The result shows that the qualitative characters of the epidermis (the shape, anticlinal walls of ordinary cells in the both side, stomatal types, trichomes) are the similar among the three populations (Fig. 4 a-f), though some differences in quantity parameters, such as stomatal size, stomatal density appear to be different between populations. This result indicates the specific stability in leaf fingerprint in the six *Rhododendron* species.

In summary, these comparative data of leaf fingerprint are significant to suggest that leaf epidermal fingerprints of *Rhododendron* have highly conserved specificity and can be considered as the key character for the classification of *Rhododendron*. For the morphological similar species, it will also be significant to combine with evidences from scanning electronic microscope (SEM) and other studies such as cytology, palynology, anatomy and molecular biology, etc. in order to arrive at a concrete delimitation of taxa.

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